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Short Communication

Role of slow oscillatory activity and slow wave sleep in consolidation of episodic-like memory in rats

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HIGHLIGHTS

- We studied sleep effects on consolidation of episodic-like memory and its components in rats.
- We confirmed that sleep following learning enhances episodic-like memory and object-place memory.
- Episodic-like memory correlated with power of slow oscillations in EEG during slow wave sleep following learning.
- Object-place memory correlated with percentage of slow wave sleep during consolidation period.

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ABSTRACT

Our previous experiments showed that sleep in rats enhances consolidation of hippocampus dependent episodic-like memory, i.e. the ability to remember an event bound into specific spatio-temporal context. Here we tested the hypothesis that this enhancing effect of sleep is linked to the occurrence of slow oscillatory and spindle activity during slow wave sleep (SWS). Rats were tested on an episodic-like memory task and on three additional tasks covering separately the *where* (object place recognition), *when* (temporal memory), and *what* (novel object recognition) components of episodic memory. In each task, the sample phase (encoding) was followed by an 80-min retention interval that covered either a period of regular morning sleep or sleep deprivation. Memory during retrieval was tested using preferential exploration of novelty vs. familiarity. Consistent with previous findings, the rats which had slept during the retention interval showed significantly stronger episodic-like memory and spatial memory, and a trend of improved temporal memory (although not significant). Object recognition memory was similarly retained across sleep and sleep deprivation retention intervals. Recall of episodic-like memory was associated with increased slow oscillatory activity (0.85–2.0 Hz) during SWS in the retention interval. Spatial memory was associated with increased proportions of SWS. Against our hypothesis, a relationship between spindle activity and episodic-like memory performance was not detected, but spindle activity was associated with object recognition memory. The results provide support for the role of SWS and slow oscillatory activity in consolidating hippocampus-dependent memory, the role of spindles in this process needs to be further examined.

Episodic memory is defined by the ability to replay in mind a past event as it happened in a specific spatio-temporal context [1,2]. Accumulating evidence suggests that sleep supports the consolidation of hippocampus-dependent memory [3–5]. This benefitting effect is primarily conveyed by slow wave sleep (SWS) and the slow oscillations [6]. It has been proposed that the slow oscillations enhance hippocampal memories by synchronizing the reactivation of respective neuronal representations to the excitable up-state [7]. The depolarizing up-state of the slow oscillation drives spindle activity originating in thalamo-cortical networks [8]. The co-occurrence of reactivations of hippocampal memories and spindles during the up-state is thought to support the formation of a

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distributed memory representations spanning across hippocampal and extra-hippocampal networks [4].

In animals, the investigation of episodic-like memory concentrates on its major feature, i.e. the binding of an event into its spatio-temporal context, as the subjective component of autonoetic consciousness cannot be addressed. Our previous studies in rats showed that a short period of sleep after encoding is critical for maintaining episodic-like memory, spatial memory and temporal memory, which are linked to hippocampal function, whereas object recognition memory did not profit from sleep [9,10]. However, while those experiments demonstrated the importance of sleep for memory consolidation, they lacked an assessment of the underlying sleep EEG. Here we set out to test the hypothesis that the enhancing effect of sleep on episodic-like memory in rats is linked to SWS and the occurrence of slow oscillatory and spindle activity during the post-encoding retention interval.

Twelve adult male Long Evans rats were kept in regulated light/dark (12 h/12 h) conditions with light onset at 6 a.m., with water and food available *ad libitum*. The experiments were performed between 8:00 a.m. and 1:00 p.m. (i.e., in the first half of the rest phase when sleep pressure is typically high). All experimental procedures were performed in accordance with the European animal protection laws and policies (Directive 86/609, 1986, European Community) and were approved by the Schleswig–Holstein state authority.

In order to characterize sleep pattern and slow oscillation activity, four screw EEG electrodes were chronically implanted under isofluorane anesthesia (two frontal electrodes AP: +2.6 mm, L: ±1.5 mm relative to Bregma and two occipital reference electrodes AP: −10.0 mm, L: ±1.5 mm). Two stainless steel wire electrodes were implanted bilaterally in the neck muscles for EMG recordings. The electrodes were fixed to the skull with cold polymerizing dental resin. At the time of recordings the electrodes were connected through a swiveling commutator to an amplifier (Model 15A54, Grass Technologies, USA). EEG and EMG signals were amplified, filtered (EEG: 0.01–300 Hz; EMG: 30–300 Hz), and sampled at the rate of 1000 Hz. After at least seven days for recovery, rats were habituated for three days to the empty open field arena (10 min per day) and immediately afterwards to the recording box (80 min per day). To test retention of episodic-like memory and its components, we used a non-stressful, one-trial based episodic-like memory (EM) task, and three additional tasks assessing spatial (object place recognition – OPR), temporal (temporal memory – TM) and item memory (novel object recognition – NOR). The behavioral procedures were described in [10]. The tasks were executed in the following order: NOR, OPR, TM, EM, with at least two days between subsequent tests. This sequence of tasks was repeated twice, with the sleep and sleep deprivation conditions alternating across tasks in a within subject design. All tasks comprised a sample phase, an 80-min retention interval, and a test phase. The sample phase for each task allowed the rat to explore two (or four) objects in the open field until it had accumulated at least 15 s of exploration for each object within an interval of 2–5 min. The retention interval was filled either with normal morning sleep, or sleep deprivation in the recording box. In the sleep condition the rats were left undisturbed. Sleep deprivation was achieved by gentle handling; if the animal displayed a sleeping posture it was aroused by tapping on the box, gently shaking the box or if necessary disturbing the sleeping nest. For the test phase, the rats were placed in the open field arena to allow exploration for 3 min.

The EM task (Fig. 1A) included two sample sub-phases which were separated by an interval of 20 min. In the first sample sub-phase four identical objects were presented (old-familiar objects). In the second sub-phase a set of four identical objects (different from those used in the first sub-phase) was presented (recent-familiar objects). In the test phase, animals were exposed to two old-familiar and two recent-familiar objects. One of the old-familiar objects and one of the recent-familiar objects was placed at the same location as in the corresponding sample phase (old-familiar stationary and recent-familiar stationary) while the other two objects were placed in new locations (old-familiar displaced and recent-familiar displaced). This arrangement allows testing directly the binding of spatial and temporal component. The interaction between spatial and temporal component effects is basically assessed by comparing exploration time for the object that is both old and displaced (i.e., the old-familiar displaced object) with exploration times for the objects for which either only the temporal component (i.e., the old-familiar stationary object) or only the spatial component (i.e., the recent-familiar displaced) is manipulated [11]. In the OPR task, two identical objects were presented in the open field during the sample phase. In the test phase the same two objects were presented with one of the objects being displaced from its original position. Relatively enhanced exploration of the displaced object indicates memory for the location of the non-displaced object. The TM task consisted of two sample sub-phases, separated by a 20-min interval. During the first sub-phase, two identical objects were presented and in the second sub-phase, two different identical objects were presented in the same locations. For the test phase one object from each sample sub-phase was presented (at the original location). Relatively enhanced exploration of the earlier presented object indicates temporal order memory. In the NOR task the sampling phase was the same as in the OPR task. In the test phase, one of the objects was replaced by a different novel object. Relatively enhanced exploration time for the novel object indicates memory for the familiar object.

Exploration behavior was analyzed offline using the ANY-maze tracking system (Stoelting Europe, Ireland). For the EM task, the time an individual rat spent exploring each object during the test phase (retrieval) was converted into an discrimination ratio binding temporal and spatial context components: [(old-familiar stationary object – recent-familiar stationary object) + (recent-familiar displaced object – recent-familiar stationary object)]/(old-familiar stationary object + old-familiar displaced object + recent-familiar stationary object + recent-familiar displaced object) [10]. For the OPR, TM, and NOR tasks discrimination ratios were based on the formula: [(novel object – familiar object)/(novel object + familiar object)], where "novel" refers to the displaced object on the OPR task, the old-familiar object on the TM task, and novel object on the NOR task; "familiar" refers to the respective other object. Sleep was scored using 10-s epochs according to standard criteria [12] (SleepSign for Animal, Kissei Comtec, Japan). Periods of waking, SWS, REM sleep and pre-REM sleep were identified. Furthermore, Fast Fourier Transformation was performed and average power during SWS was then calculated for the 0.85–2.0 Hz slow oscillation (SO) range. Sleep spindles were detected based on the algorithm used by [13]. For the TM and EM tasks, data from one rat were discarded due to the loss of the implant, and for the EM task, an additional rat was discarded due to technical failure, thus resulting in a final $n = 12$ for the NOR and OPR tasks, $n = 11$ for the TM task and $n = 10$ for the EM task. For statistical analyses SPSS 21.0 (IBM, Armonk, USA) software was used.

In the EM task, sleep during the 80-min post-encoding retention interval distinctly improved performance in the test phase, as compared with the sleep deprivation condition (Student's *t*-test: $t(9) = 2.67$, $p = 0.026$, Fig. 1B). In fact, only when rats had slept during the retention interval did they achieve discrimination ratio above chance level (One-sample t-tests: sleep: $t(9) = 2.74$, $p < 0.05$, sleep deprivation: $t(9) = -0.38$, $p = 0.71$). In addition, exploration time was analyzed separately for the four objects of the EM task using a $2 \times 2 \times 2$ repeated measures ANOVA with the factors "retention condition" (sleep vs. sleep deprivation), "temporal component" (old-familiar vs. recent-familiar) and "spatial

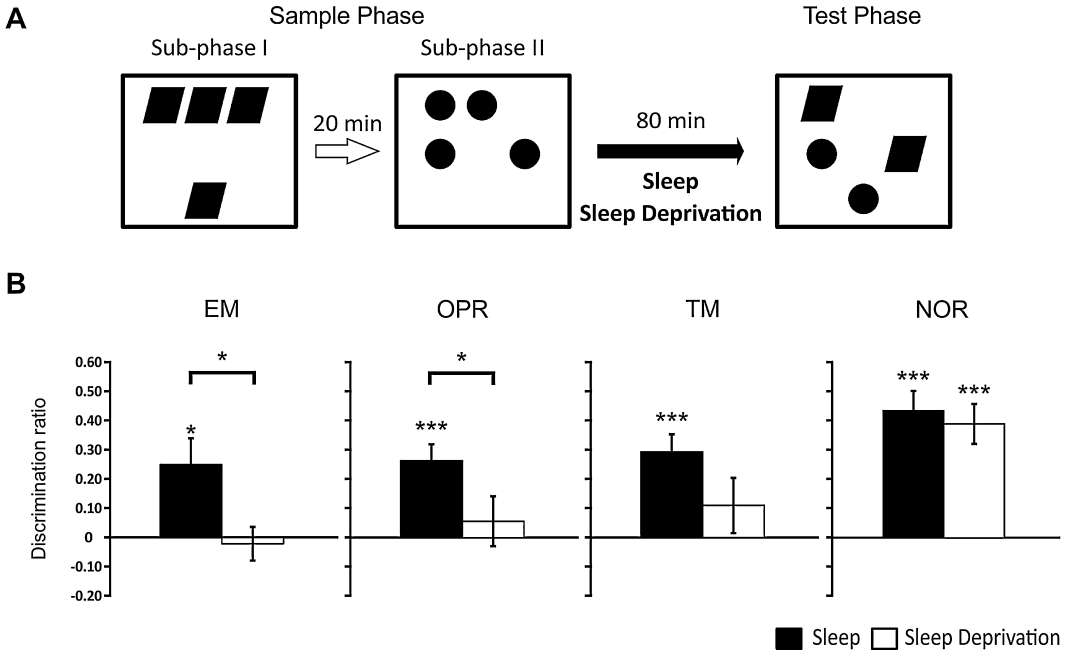

Fig. 1. (A) Schematic of EM task showing example arrangements of objects during sample and test phases. (B) Discrimination ratios indicating memory performance during the test phase in EM task, OPR task, TM task and NOR task. Means (±SEM) discrimination ratios are indicated for the sleep (black bars) and sleep deprivation (empty bars) conditions. Asterisks on top of the bars indicate significance in comparison with chance level, asterisks above horizontal lines indicate significance for pairwise comparisons between conditions (*$p < 0.05$, ***$p < 0.001$).

component" (displaced vs. stationary). Consistent with our hypothesis of a sleep effect on EM performance, this analysis revealed a significant 3-way interaction between retention condition x spatial component x temporal component ($F(1,9) = 20.4$, $p = 0.001$). Sub-ANOVAs run separately for the sleep and sleep deprivation conditions confirmed the presence of episodic-like memory in the sleep condition as reflected by high significance for the spatial component × temporal component interaction ($F(1,9) = 18,7$, $p = 0.002$). The same interaction was also significant in the sleep deprivation condition ($F(1,9) = 7.7$, $p = 0.022$). However, this interaction mainly originated from a distinctly enhanced exploration time for the old-displaced object, i.e., a pattern diverging from that of normal episodic memory binding (see [10]). In the OPR task, the memory was also better after the sleep retention interval than after sleep deprivation ($t(11) = 2.27$, $p = 0.044$, Fig. 1B). Only after the sleep retention interval did discrimination ratio significantly differ from chance (sleep: $t(11) = 4.63$, $p < 0.001$; sleep deprivation: $t(11) = 0.66$, $p = 0.53$). In the TM task, the memory improvement in the sleep condition compared to the sleep deprivation condition did not reach significance ($t(10) = 1.30$, $p = 0.22$). The discrimination ratio after the sleep retention interval was distinctly above chance ($t(10) = 4.91$, $p < 0.001$), whereas after sleep deprivation was not ($t(10) = 1.17$, $p = 0.27$). Performance in the NOR task did not profit from sleep during the retention interval ($t(11) = 0.49$, $p = 0.64$, Fig. 1B). Discrimination ratios in both the sleep and sleep deprivation conditions were significantly above chance level (sleep: $t(11) = 6.38$, $p < 0.001$; sleep deprivation: $t(11) = 5.74$, $p < 0.001$).

Total object exploration times during the sample phase did not differ significantly between the sleep and sleep deprivation condition for any of the tasks. Total exploration did also not differ between the retention conditions for the test phases of the NOR and TM tasks. On the OPR and EM tasks, total exploration time during the test phase was longer after sleep than after sleep deprivation ($t(11) = 2.75$, $p = 0.019$ and $t(9) = 3.05$, $p = 0.014$). However, we did not find significant correlation between total exploration time during the test phase and memory performance on any one of the tasks ($p > 0.05$). Furthermore, the notion that sleep effects on memory do not result from an unspecific facilitation of exploration is supported by our previous findings showing an enhancing effect of sleep on memory in the absence of any change in exploration times [14].

Total sleep time (35.45 ± 1.33 min) and sleep architecture during the sleep retention interval did not differ between the tasks ($p > 0.5$, Supplementary Table 1). EM task performance was associated with increased slow oscillatory EEG activity (0.85–2.0 Hz; Pearson $r = 0.64$, $p = 0.047$, Fig. 2A). Contrary to our expectation, spindle counts or density were not correlated with retention on the EM task ($p = 0.63$). OPR performance correlated positively with percentage of SWS within total sleep ($r = 0.750$, $p < 0.01$, Fig. 2B). NOR performance correlated negatively with the percentage of SWS ($r = -0.629$, $p < 0.05$) and showed a significant association of spindle counts during SWS ($r = 0.63$, $p = 0.027$, Fig. 2C). Supplementary Table 2 provides correlations between sleep parameters and performance in the four tasks.

We further examined whether the sample phase experience affected subsequent retention sleep, compared with baseline sleep (i.e., sleep after the last of the three habituation sessions). Except for a trend toward increased 2.0–4.0 Hz delta power during SWS ($F(3, 24) = 3.07$, $p = 0.07$), these analyses did not reveal any systematic change in sleep following the sample phase.

Behavioral effects of sleep largely replicated observation of our previous studies, indicating a critical sleep-dependency of intermediate-term episodic (EM task) and spatial (OPR task) memory [10,14,15]. NOR performance was not affected by sleep, again consistent with our previous findings. The observed enhancement of TM performance by sleep was not significant, in contrast to our previous report, suggesting less robust effects of sleep in this task. We suspect this negative outcome is owed partly to the general difficulty to sensitively measure purely temporal memory at the behavioral level in animals.

While we did not observe an effect of sleep vs. sleep deprivation in the NOR task, in several previous papers such effect was reported [16,17]. In contrast to these studies, we used rats rather than mice and employed a distinctly shorter retention interval. In addition, the high performance of the sleep deprived condition in our study

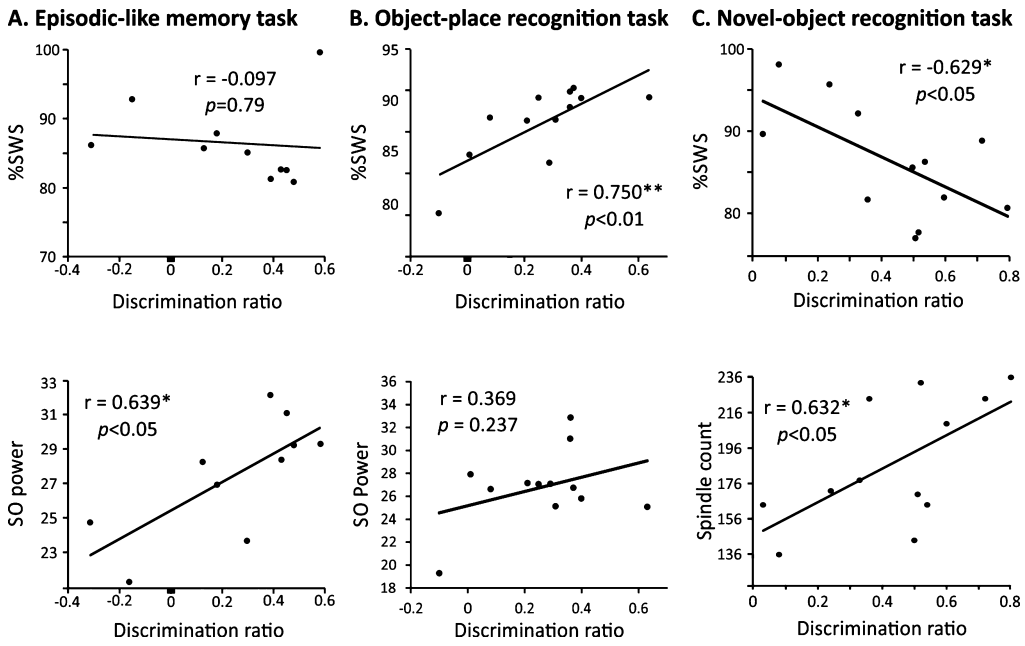

Fig. 2. (A) Correlations (Pearson) between EM performance and the percentage of SWS and EEG power in the slow oscillation frequency band (0.85–2.0 Hz). (B) Correlations between OPR performance and percentage of SWS and EEG power in the slow oscillation frequency band. (C) Correlations between NOR performance and the percentage of SWS, and spindle counts during SWS (*$p < 0.05$, **$p < 0.01$).

could have made it harder to detect a sleep induced improvement. Nevertheless, our data show unambiguously that the consolidation of this task did not require sleep. Considering the notion that unlike the other tasks, which are hippocampus dependent, the role of hippocampus in NOR appears limited [18, but see 19], our findings may corroborate the view that sleep preferentially benefits hippocampus-dependent memory [20,21].

In support for our main hypothesis that slow oscillatory activity associated with SWS is important for consolidation of hippocampus-dependent memories, we observed that (i) performance on the episodic-like memory task was positively correlated with slow oscillation power and (ii) performance on the object-place recognition task was positively correlated with percentage of SWS during retention sleep. Although correlation analyses strictly speaking cannot provide information on causality, these findings add novel evidence in support of a relationship between slow wave activity and memory performance.

As the slow oscillations hallmark SWS, the correlation of EM performance with slow oscillatory EEG power in the absence of a similarly significant correlation with the percentage of time spent in SWS might surprise. However, previous studies have shown that slow oscillation amplitude can change independently of the time spent in SWS (e.g. [22]). The correlation with slow oscillation power was revealed only for performance on the EM task, which might suggest a particular functional specificity of this oscillation for integrating spatio-temporal context into an episodic memory. The observed correlation between episodic memory formation and slow oscillatory activity during retention sleep converges with studies mostly in humans (e.g. [6]).

Contrary to our expectation, we did not observe an association of spindle activity with memory performance on the EM task, which diverges from previous studies in humans and rats (e.g. [13,23,24]). The lacking link between spindle activity and episodic-like memory performance may relate to the rather short retention interval. Assuming that spindles benefit consolidation primarily by supporting the transfer of reactivated hippocampal memory information to extra-hippocampal sites, and assuming that this redistribution of hippocampal representations is a more gradual process, retrieval testing after a retention interval of only 80 min might reflect primarily strengthening effects of sleep-associated reactivations on the hippocampal representation per se, rather than effects originating from the redistribution of the memory representation.

We believe that stress and tiredness in our protocol did not impair memory performance after sleep deprivation. The period of sleep deprivation was relatively short, and gentle handling procedures applied during such short periods do not produce substantial increases in corticosterone concentrations [25,26]. Furthermore, in our previous experiments which directly controlled for possible stress effects by testing the rats after retention periods of spontaneous wakefulness in the late evening hours (i.e., during their natural active phase), we observed an identical pattern of memory performance [9,10].

An 80-min retention interval was chosen in this and our previous studies because spatial memory in the OPR task may degrade across longer intervals and cannot be reliably assessed (e.g. [16]). However, with the short duration of the retention period we might have only probed effects of sleep on intermediate-term memory ranging between minutes and hours. Therefore, future studies have to scrutinize how these effects of sleep translate into the formation of long-term memories.

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Appendix A. Supplementary data

Supplementary data associated with this article can be found, in the online version, at http://dx.doi.org/10.1016/j.bbr.2014.09.008.

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